Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

COMPLEMENT FIXATION WITH ACID-FAST BACTERIA

II. IN LEPROSY

J. V. COOKE

From the Department of Pathology and Bacteriology, University of California, and the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York

During the past decade, the study of leper serums with various antigens by the fixation test has interested a number of observers in various places. A correct comparison of the results of the many observations is rendered difficult because, not only has the technic of the reaction varied, but the kind of antigen used in the tests has been modified in numerous ways. In general, the recorded observations may be divided into three groups: (1) Wassermann reactions, (2) complement fixations with the use of tissue extracts, and (3) tests with bacterial antigens. It is rather striking that no matter what kind of antigen has been used, a certain proportion of positive results has been obtained. Some have reported a high percentage of positive tests, while others have obtained only a relatively small percentage. Apparently, all have felt that fixation antibodies are present in the leper serum, and the variety of antigens employed is an evidence of the search for the one that would constantly react with all or a large proportion of the cases.

From time to time partial and frequently inaccurate compilations of the work of investigators in this field have been made, but no comprehensive collection or analysis of cases occurs in the literature. Since it was found possible in the course of this investigation to review all of the original publications, it was thought desirable to tabulate the results of this study which comprises all the previously reported work on fixation in leprosy. Some care has been taken to make the bibliography complete, and it seems certain that this report contains all the reactions reported with the possible exception of certain isolated cases included with work not closely related and consequently extremely difficult of access. An additional reason for tabulating the cases is to illustrate the relation of my own studies to those of other investigators and to point out some rather fundamental features in fixation reactions with leper serums that have apparently not been

Received for publication July 25, 1919.

understood previously. In some instances a regrouping of the cases reported by certain authors has been rendered necessary by the arrangement adopted in the tables. Occasionally, I have taken the liberty of making certain slight alterations in authors' figures that seemed quite justified. For example, serums that were anticomplementary have been omitted entirely even though they had been included as "positive" by the author. Again, cases called "weak positive" have been included under positive while those reported as "very faint inhibition," "very faintly positive," "almost complete hemolysis," or "doubtful" have been placed with the negative cases.

One criticism that applies to a very large part of the work is the lack of proper controls. With regard to the Wassermann reactions this omission is probably less important, since this test has been performed on such a large scale on so many diseases. Very few authors made any but casual mention of any series of control serums tested, although it is possible that such omissions were merely due to the fact that it was not thought necessary to mention them. With regard to the other antigens, however, one can only assume that the very few control serums mentioned were the only ones tested. Obviously, the specificity of the reaction should have been controlled, not only by the use of a considerable number of serums from diseases other than leprosy, but also by the use of other antigens prepared from unrelated tissues or bacteria. This procedure has been carried out by only a very few.

THE WASSERMANN REACTION IN LEPROSY

The antigens used in Wassermann reactions have included alcoholic extracts of human and guinea-pig heart, normal and syphilitic liver, syphilitic fetus and the acetone insoluble lipoid antigen of Noguchi. Since it is generally agreed that these various methods of preparing lipoidal antigens have little effect on the result of the reactions, no attempt has been made to group the cases according to the antigen used.

The presence of fixation antibodies in leper serum with the various lipoid antigens used in the Wassermann reaction has been rather a pathologic curiosity. As may be seen by table 1, 42 investigators have made more or less extended studies on this phenomenon. One or two writers have been interested to determine whether leprosy causes a positive Wassermann reaction or whether a relatively high percentage of lepers have syphilis, but unfortunately there are few reported studies of unselected control groups of cases without leprosy to determine the percentage of latent syphilis. As a rule, however, the number of lepers giving a positive Wassermann is high enough to justify the conclusion that leprosy alone may cause the blood to contain substances that give fixation with lipoidal antigens. That the proportion of leper serums giving a positive Wassermann test is probably not so great as is generally considered is indicated by a summary of all the reported cases. In all there

TABLE 1
WASSERMANN REACTION IN LEPROSY

Name of Author	Year	All Cases Leprosy		Nodular and Mixed		Anesthetic Cases		Remarks
		Number	Per Cent. Positive	Number	Per Cent. Positive	Number	Per Cent. Positive	
1. Eitner	1908	1	100					
2. Wechselmann	1908	1	100	..	..	..	..	Fixation also with 1 per cent. lecithin
3. Slatinéanu and Daniélopolu	1908	21	76	..	..	..	..	Spinal fluid gave positive reaction in 6 cases
4. Masslakowetz	1908	16	56	..	..	..	..	Controls: 2 syphilitics positive, 1 normal, negative
5. Jundell, Almkvist and Sandman	1908	23	35	12	42	11	27	2 other cases anticomplementary; 1 case suspected leprosy negative
6. Bruck and Gessner	1909	10	50	7	71	3	0	
7. Castelli	1909	1	100					
8. Ehlers and Bourret	1909	5	60	..	..	..	..	39 other serums were partially anticomplementary
9. Eliasberg	1909	50	56	31	81	19	16	
10. Fleming	1909	2	100					
11. Frugoni	1909	4	75	..	..	..	..	1 positive case has clinical syphilis
12. Inouye, Ito and Nakano	1909	39	38	23	10	16	31	
13. Noguchi	1909	10	70					
14. Serra	1909	17	76	14	93	3	0	
15. Åkerberg, Almkvist and Jundell	1910	14	15	5	40	9	0	Supplementary report to (5)
16. Babes and Busila	1910	9	89	8	100	1	0	Used 0.2 to 0.8 c c of serum
17. Baermann and Netter	1910	140	56	124	65	16	50	In 100 normal (Chinese) controls 25 per cent. positive
18. Fox	1910	60	57	38	82	22	19	
19. Meier	1910	31	45	19	63	12	17	
20. Montesano and Sotiriadès	1910	48	48	36	86	12	17	
21. Nishiura	1910	136	32	67	46	69	19	
22. Steffenhagen	1910	5	60					
23. Thomsen and Bjarnhjedinson	1910	50	22	31	36	19	0	53 per cent. were positive with active serum
24. Bloomberg	1911	21	14					
25. Duval and Gurd	1911	27	81	22	91	5	40	
26. DeHaan and Grijns	1911	18	39	6	33	12	42	2 of the negative cases were "doubtful"
27. Krefting	1911	1	100	..	..	..	..	Negative with another antigen
28. Müller and Suess	1911	6	100	6	100			
29. Schmitt	1911	7	71	5	80	2	50	
30. Bates	1912	42	47					
31. Photinos and Michaélidès	1912	204	56	104	75	100	38	
32. Racamora	1912	19	74	17	82	2	0	Salvarsan had no effect on reaction
33. Schüffner	1912	32	26	32	26			
34. Wills	1912	10	40					
35. Clegg	1913	24	46	..	..	..	..	All positives were nodular type
36. Kritchewsky and Bierger	1913	17	71	11	100	6	17	
37. Lagane and Columbier	1913	7	71	3	67	4	75	
38. Schmitter	1914	25	80					
39. Fletcher	1915	100	22	44	27	56	18	10 per cent. of 110 cases malaria and beriberi positive
40. Mathis and Baujean	1915	41	2	40	2	1	0	Positive case had clinical syphilis
41. Nakajo and Asakura	1915	23	69	18	74	5	60	
42. Sordelli and Fischer	1916	80	61	..	..	..	..	1 case anticomplementary not included; 103 cases not leprosy gave 13 positives
Totals	......	1,397	50	723	60	405	25	

have been collected 1,397 cases of leprosy of which 705 or 50.4% have given a positive reaction. The general impression that one gets from statements in textbooks and articles discussing the Wassermann reaction in leprosy is that the percentage of positive results is considerably higher than this figure, although no previous analysis of such a large series has been attempted.

The relation of the type of the disease to the presence of a positive reaction may be determined by an analysis of those cases in which the observer classified the cases studied. In this, cases of nodular or skin leprosy have been grouped with those having evidences of the disease both in the skin and nerves —the so-called mixed type—and the purely nerve or anesthetic cases have been grouped separately. Seven hundred and twenty-three cases of nodular and mixed leprosy have given 433 positive reactions, or 60%, while of the 405 cases of anesthetic leprosy, only 103, or 25.4%, were positive. That these figures are comparable to the large group of all the reported cases is shown by combining them—1,128 cases with 536, or 47.5%, positive.

To summarize approximately these results, it will be seen that half of all the cases of leprosy studied have given a positive Wassermann reaction, while three fifths of the nodular and mixed cases and one fourth of the purely anesthetic type have reacted positively. Since this fixation is a lipoidal reaction and admittedly nonspecific, no discussion of its significance will be made here. It is obvious, however, that while a certain proportion of the positive fixations may be accounted for by a coincident syphilitic infection, the percentage is entirely too high to be explained by this factor alone, and we must conclude that infection with the Hansen bacillus may cause the appearance in the blood of substances capable of giving a positive Wassermann reaction, and that these substances are more frequently present in cases with skin lesions, than in cases with nerve lesions alone.

TISSUE EXTRACT ANTIGENS

In reviewing the results of complement fixation in leprosy with antigens made from tissue extracts, two groups have been made: (1) alcoholic, and (2) aqueous extracts of tissues. The first group is tabulated in table 2. The preparation of these antigens is essentially similar to that used for Wassermann antigens except that lepromas were extracted by alcohol and in a few instances the ether soluble substances of such tissue were used also. Such antigens are evidently essentially lipoidal and their use should give results similar to those obtained in the Wassermann series. By referring to the table, it will be seen that of the 210 cases tested with these antigens 110 or 52% were positive. In almost all of these the type of disease is stated and 82 (75%) of the 109 tubercular and mixed cases, with only 23 (25%) of the 91 purely anesthetic cases were positive. Even this relatively small series shows a rather close relation to the percentages found by using the usual lipoidal Wassermann antigen (table 1), and there seems no doubt that fixation with leper serums and alcoholic extract of leproma as antigen has no more specificity than the Wassermann reaction in similar cases. This is further shown by the work of **Frugoni** and Pisani, who obtained somewhat better results with the use of alcoholic extracts of sarcoma and carcinoma as antigen than with the leproma extract.

TABLE 2

COMPLEMENT FIXATION IN LEPROSY WITH TISSUE EXTRACT ANTIGENS (ALCOHOLIC)

Author	Year	Antigen Alcoholic Extract of	All Cases Leprosy		Nodular and Mixed		Anesthetic Cases		Remarks
			Number	Per Cent. Positive	Number	Per Cent. Positive	Number	Per Cent. Positive	
1. Babes and Busila*	1909	Lepromas†	5	100	5	100	....	....	1 of 3 cases of syphilis was positive
Babes and Busila	1909	Normal skin†	5	0	5	0	....	....	3 cases of syphilis negative
2. Frugoni and Pisani*	1909	Lepromas	10	50					
Frugoni and Pisani	1909	Sarcoma	11	73					
Frugoni and Pisani	1909	Carcinoma	11	64					
3. Récio...........	1909	Lepromas	18	78	14	92	4	25	1 of anesthetic cases "doubtful"; 2 cases syringomyelia negative
4. Nishiura........	1910	Leprous liver	135	44	67	64	68	24	4 of 10 cases of syphilis were positive
5. Schmitt........	1911	Rat leprosy tissues‡	7	100	5	100	2	100	Of 5 cases of syphilis 3 were positive; 7 cases various diseases neg.
6. Jeanselme......	1912	Alcoholic ether extrace of lepromas	9	56	5	80	4	25	1 normal person and 2 out of 8 syphilitics gave positive reactions
7. Jeanselme and Vernes	1915	Same	7	57	4	100	3	0	32 of 40 cases of syphilis were positive; 21 normal persons negative
8. Nakajo and Asakura	1915	Lepromas	19	58	9	89	10	30	
Totals..........	......	....	210	52	109	75	91	25	

* Only tests with leproma antigen included in totals.
† Ether extract used.
‡ Acetone insoluble fraction (Noguchi).

The aqueous tissue extracts used as antigen were made by grinding fresh or dried lepromas with salt solution, and allowing the coarser particles to sediment. The supernatent slightly turbid fluid was preserved with a small amount of phenol or other antiseptic and used as antigen after testing its anticomplementary properties. Any variation of this technic used did not essentially alter the character of the antigen with the possible exception of two observers who used antiforminized lepromas as antigen. The results with such extracts are given in table 3. Two hundred and three or 66% of the 309 cases of leprosy gave positive reactions. One hundred and forty-four cases of nodular or mixed leprosy showed 121 or 84% positive while 56 or 41% of the 137 cases of the anesthetic type reacted positively. Among the relatively few control cases of other diseases tested with these antigens, certain cases of syphilis and of tuberculosis have reacted positively. The probable explanation of these reactions will be discussed later.

TABLE 3

COMPLEMENT FIXATION IN LEPROSY WITH TISSUE EXTRACT ANTIGENS (AQUEOUS)

Author	Year	Antigen Aqueous Extract of	All Cases Leprosy		Nodular and Mixed		Anesthetic Cases		Remarks
			Number	Per Cent. Positive	Number	Per Cent. Positive	Number	Per Cent. Positive	
1. Eitner..........	1906	Lepromas	1	100	1	100			
2. Eitner..........	1908	Lepromas	1	100					
3. Gaucher and Abrami	1908	Lepromas	9	100	8	100	1	100	16 cases syphilis and 8 cases syringomyelia negative; 3 of 10 cases tuberculosis positive
4. Slatinéanu and Daniélopolu	1908	Lepromas	26	92	....	....	....	....	11 of the 19 cases gave positive reaction with spinal fluid
5. DeHaan........	1909	Lepromas	16	9	5	20	11	9	11 of the cases gave "doubtful" reactions
6. Pasini..........	1909	Lepromas	3	67	3	67			
7. Serra...........	1909	Lepromas	17	88	14	100	3	33	
8. Serra...........	1909	Normal organs	17	76	14	93	3	0	Control
9. Sugai...........	1909	Lepromas	2	100	1	100	1	100	5 other cases anticomplementary
10. Åkerberg, Jundell and Almkvist	1910	Lepromas	28	34	12	67	16	13	11 of 17 cases syphilis were positive; 6 of 11 cases syphilis and gonorrhea with negative Wassermann gave positive reaction
11. Babes and Busila	1910	Lepromas	9	100	7	100	2	100	Used 0.2 to 0.6 c c serum
12. Nishiura........	1910	Lepromas	141	65	67	82	74	50	Slightly higher per cent. of positive reactions with aqueous extract of leprous liver antigen
13. Steffenhagen....	1910	Antiforminized lepromas	5	80	5	80			
14. Thomsen and Bjarnhjedinson	1910	Lepromas	19	16	3	100	16	0	Same results with normal skin antigen; 3 cases syphilis positive
15. Biehler and Eliasberg	1911	Antiforminized lepromas	18	94	8	100	10	90	
16. Clegg, McCoy and Hollman	1914	Lepromas	13	85	10	90	3	67	4 normal controls negative; 1 of 2 cases pulmonary tuberculosis pos.
Totals.........	......	....	308	66	144	84	137	41	

The proportion of positive fixation, therefore, in all types of leprosy, is higher when aqueous extracts of lepromas are used than when alcoholic extracts of leprous or other tissues are employed as antigen. Such aqueous leproma extracts, beside certain lipoidal and other substances probably giving a nonspecific fixation, must contain in addition enormous numbers of acid-fast bacilli since the skin nodules of leprosy are usually filled with micro-organisms. It is quite likely that these Hansen bacilli add a definite specificity to the antigen and may be largely responsible for the higher percentages. The results of Steffenhagen and Biehler and Eliasberg, who obtained more than 80% of positive reactions by using antiforminized lepromas as antigen, support this view, since such an antigen contains less nonspecific tissue juices and relatively more acid-fast bacilli. On the other hand, Serrà and Thomsen and Bjarnhjedinson obtained almost as good results with aqueous extracts of normal organs as with lepromas, although the latter observers' figures are unusually low.

TABLE 4
Complement Fixation in Leprosy with Bacterial Antigen (Tuberculin)

Author	Year	All Cases Leprosy		Nodular and Mixed		Anesthetic Cases		Remarks
		Number	Per Cent. Positive	Number	Per Cent. Positive	Number	Per Cent. Positive	
1. Slatinéanu and Danielopolu	1908	19	58					
2. Frugoni and Pisani	1909	10	20					
3. Babes and Busila	1910	9	89	7	100	2	50	
4. Meier, G.	1910	9	89	9	89			
5. Steffenhagen	1910	5	60	5	60			
6. Moellers	1913	32	63					
7. Nakajo and Asakura	1915	38	63	23	79	15	33	1 other case anticomplementary
8. Chijo and Asakura	1916	10	70					
9. Sordelli and Fischer	1916	48	83	....	....	....	....	4 other cases anticomplementary
Totals	......	180	68	44	84	17	35	

BACTERIAL ANTIGENS

The cases in which bacterial antigens have been used have also been divided into two groups: (1) those tested with tuberculin as antigen, and (2) those in which bacillary emulsions have been used. The first group is tabulated in table 4. Apparently Koch's "old tuberculin" was used in most instances. Of 180 cases so tested, 123, or 68%, were positive. The type of leprosy was mentioned in only about a third of the cases, 44 being of the nodular or mixed variety and 17 anesthetic; 84% of the former and 35% of the latter were positive. Tuberculin, then, seems to act as well, or possibly slightly better, than aqueous extracts of lepromas as antigen.

The use of bacillary emulsion antigens has been limited to a relatively small number of cases which are tabulated in table 5. Suspensions of acid-fast bacilli were used in every instance. Several investigators have used tubercle bacilli, while others have used strains of acid-fast organisms isolated from lepers, but there has been relatively little variation in the results obtained with the use of the several organisms employed. Currie and Clegg, for example, tested the serums of four lepers with antigens prepared from several strains of acid-fast bacilli isolated from lepers, as well as extracts and emulsions of B. smegmatis and B. margarin, two saprophytic acid-fast bacilli. Their results were somewhat irregular, all organisms giving fixations of varying degree with all the cases tested and they concluded that they were unable to differentiate between the various acid-fast bacilli in this manner. Duval and Gurd, using an acid-fast organism isolated by the senior author as antigen, obtained a large percentage of positive fixations in lepers and an equally large percentage in syphilitics, both groups giving a positive Wassermann test. In other words, the emulsion of acid-fast bacilli was indistinguishable in its antigenic action in syphilis and leprosy from the usual lipoidal antigens. Steffenhagen noted that 2 out of 5 cases of leprosy tested by him gave positive fixation using an emulsion of typhoid bacilli as antigen. Wills found that several

TABLE 5

COMPLEMENT FIXATION IN LEPROSY WITH BACTERIAL ANTIGENS (BACILLARY EMULSIONS)

Author	Year	Antigen Cultures of Bacillus	All Cases Leprosy		Nodular and Mixed		Anesthetic Cases		Remarks
			Number	Per Cent. Positive	Number	Per Cent. Positive	Number	Per Cent. Positive	
1. Frugoni and Pisani	1909	Tuberculosis	11	73					
2. Mezinescu	1909	Rat leprosy	24	92	18	100	6	67	1 other case anticomplementary
3. Steffenhagen	1910	Tuberculosis	5	60	5	60	....	....	2 cases gave positive reaction with antigen of typhoid bacilli
4. Currie and Clegg	1910	Various acid-fasts	4	100	3	100			
5. Duval and Gurd	1911	Leprae of Duval	27	85	22	95	5	40	7 normal serums gave negative reactions but 22 of 23 cases of syphilis gave positive reactions
6. Bayon	1912	Leprae of Kedrowsky	1	100					
7. Wills*	1912	Human tuberculosis	9	67					
	1912	Bovine tuberculosis	11	64					
	1912	"Urine"	9	56					
	1912	Blindworm	9	44					
	1912	Timothy grass	9	56					
8. Kritchewsky and Bierger*	1913	Human tuberculosis	8	88	7	86	1	100	
	1912	Leprae of Kedrowsky	28	68	20	90	8	13	Using 0.8 c c, 5 of the negative serums became positive
	1913	Leprae of Duval	28	7	20	10	8	0	5 nodular and 2 nervous cases gave "feebly positive" reaction
	1913	Korn I	11	0	11	0	....	....	2 cases gave "feebly positive" reaction
	1913	Typhosus	11	0					
Totals	......	....	89	83	55	93	12	58	

* Only the tests using human tubercle bacilli antigen are counted in the totals.

strains of acid-fast organisms acted almost equally well as antigen with leper serums, but Kritchewsky and Bierger conclude that Kedrowsky's strain of B. leprae is superior in its antigenic properties to the other organisms studied and were unable to obtain fixation in leper serum with a B. typhosus antigen.

On the whole, bacillary emulsions have somewhat better antigenic properties than the other antigens used in leprosy, although the group of cases studied in this manner is small.

In table 6, the totals of all the groups are summarized and the proportion of positive fixations indicated. It is rather striking to note the gradually increasing percentage of positive results with the different types of antigens, the Wassermann antigen giving the lowest percentage and the bacillary emulsion antigens the highest.

Certain features of the analysis of the results of complement fixation in leprosy with various antigens may be briefly stated.

TABLE 6

SUMMARY OF POSITIVE FIXATION TESTS IN LEPROSY; ALL ANTIGENS

Table	Antigen	All Cases Leprosy with per Cent. Positive	Total Skin Cases with per Cent Positive	Total Nerve Cases with per Cent. Positive
1	Wassermann..................	(1,397) 50	(723) 60	(405) 25
2	Alcoholic leproma extract....	(210) 52	(109) 75	(91) 25
3	Aqueous leproma extract....	(308) 66	(144) 84	(137) 41
4	Tuberculin....................	(180) 68	(44) 84	(17) 35
5	Bacillary emulsions..........	(89) 83	(55) 93	(12) 58

The serum in leprosy contains fixation bodies that can be recognized in about half the cases by the use of lipoidal antigens. When extracts or emulsions of acid-fast organisms are used as antigen, positive fixation can be obtained in a considerably larger percentage. With all antigens there is a striking difference between the number of cases of leprosy with skin lesions that give a positive fixation when compared with the cases having nerve lesions alone. The relatively smaller number of the latter cases giving positive reactions may be due to the absence of fixation substances in many of these cases, but possibly is an evidence of a lower concentration of such substances in the serum.

The most fundamental and serious criticism to which all the work is open, is with regard to the apparent lack of specificity of such fixation tests. With lipoidal antigens such as are used in the Wassermann reaction, the fixation test in leprosy is certainly no more specific than in syphilis. Alcoholic and aqueous extracts of leprous tissues belong to the same group and are open to the same objection since apparently aqueous extracts of tumors have equally good antigenic qualities. Probably the chief interest centers in the use of bacterial antigens since if specificity can be shown in the reaction, these antigens should offer a better opportunity for its demonstration than any others now available. The absence of suitable controls of tests in other diseases in the work with tuberculin as antigen renders this material of little value in the present discussion. Regarding the bacillary emulsion antigens, however, it has been noted that, together with tubercle bacilli and organisms isolated from lepers, other acid-fast bacilli act almost equally as well as antigen; that emulsions of typhoid bacilli have antigenic properties with some cases of leprosy; and that many cases of syphilis react with the bacillary emulsions as antigen. All these factors, then, throw doubt on the specificity of the fixation reactions in leprosy.

In the experimental work in the following portion of this paper, a study has been made of the fixation substances in leper serums with various acid-fast antigens by a method resembling that employed with immune rabbit serums. The specificity of the reaction has been investigated by making quantitative determinations of such antibodies with acid-fast antigens, with antigens made from nonacid-fast organisms, and with a lipoidal (Wassermann) antigen.

In the previous paper a quantitative study was made of the fixation antibodies in rabbits immunized with various members of the acid-fast group of bacteria as antigens. It was shown that in such immune serums there is a fixation antibody which reacts with many acid-fast bacteria, and that there is a tendency for certain organisms to form subgroups. This reaction is specific for this group, and may be termed acid-fast fixation. It was noted also that rabbit serums of high titer contained bodies which, in lower dilutions only, gave a nonspecific fixation with antigens made from various nonacid-fast bacteria. This nonspecific fixation is demonstrable in other serums of high titer, and may be a variety of sensitization to all bacterial protein, or associated with the lipoidal material of bacterial cells.

The present paper consists of a study of the fixation properties of a series of leper serums with acid-fast bacterial antigens. Although it may not be generally conceded that the specific cause of leprosy has been grown artificially, the disease is certainly an infection by an acid-fast organisms, and the serum might be expected to contain fixing substances which would react with antigens of acid-fast bacteria — an acid-fast fixation.

The difficulty in securing a large series of leprosy cases in the United States unless one is situated near one of the endemic foci, is obvious. However, the chief purpose in making this study was to determine the presence in leper serums of fixation antibodies for acid-fast antigens, and to compare the concentration of such antibodies in cases of different clinical types and severity. It has been possible to do this satisfactorily with the relatively small series of cases at my disposal.

METHODS

The material for study was obtained from 15 lepers from the Louisiana Leper Home through the cooperation of Dr. Ralph Hopkins of New Orleans, and from 5 cases from the University of California Dispensary through the kindness of Dr. Howard Morrow of San Francisco. The blood was obtained in the usual way by venous puncture, and the serum sealed in ampules, inactivated and kept in the icebox until used. Some of the serums were tested immediately, but others were kept for several months before the reactions were done. In none did any anticomplementary action develop. Each serum was reinactivated for 5 minutes immediately before use in the tests. This lack of anticomplementary action was attributed to the fact that the serums were sterile, sealed and kept at icebox temperature. That such serums may be kept for considerable periods with little deterioration is indicated by the fact that two serums reacting positively were retested after having been kept for 3 years with practically identical results.

The technic of the fixation test has not varied from that described previously in connection with the second series of experiments with immune rabbit serums. The antigens used were the same as those employed in the tests of immune rabbit serums reported earlier in another paper, and the amounts used in each test were identical with those used in the same tests. Unfortunately, the amount of serum available did not permit the testing of each serum with every antigen, so that certain antigens were necessarily omitted from the tests for lack of material. Every serum, however, was tested with several antigens. A simple lipoid antigen was also used in testing each of the serums. This was so-called simple alcoholic antigen such as is used in the Wassermann reaction, and was made by extraction of beef heart with ethyl alcohol in the usual way. The dose used was less than half the anticomplementary amount, and contained at least five antigenic units when tested with syphilitic serum. All patients' serums were titrated with a constant amount of antigen in making the tests. Starting with 0.2 c c of serum, gradually decreasing amounts were used until the minimal fixing dose was determined. Controls for anticomplementary action of double the antigen dose, and of the largest amount of serum used, were always included.

In table 7 is shown the minimal fixing dose of each of the various leprosy serums tested with several acid-fast antigens, and the lipoidal antigen. Some of the cases were tested also with similar antigens of

B. typhosus and a diphtheroid bacillus. The cases, a brief clinical summary of which will be found at the end of the paper, were numbered after the tests were performed in order to simplify the table. No tests of normal or control serums are shown, although many normal individuals and persons suffering from other diseases have been examined. These will be given in the following paper. It is sufficient to state here that other serums do not react with these acid-fast antigens except those from patients with tuberculosis.

TABLE 7

Complement Fixation with Leprosy Serums and Various Antigens

Antigens	Minimal Fixing Dose of Serums from Leprosy Cases in c c																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
B. leprae — Duval (chrome)	0.002	0.008	0.01	0.05	.....	0.03	0.1	....	....	0	...	0	0	...	...	0	...	0.2		
B. leprae—Levy	0.004	0.03	0.03	.....	.....	0.05	....	....	....	....	...	...	...	...	...	0.2				
B. leprae—B	0.001	0.004	0.006	.....	0.1	0.03	....	0.05	0.05	0.2	...	0	0	0.2	...	0	0	...	0	0
B. leprae—F	0.001	0.006	0.03	.....	.....	0.05	0.1	0.1	....	....	0.1	0	0.1	0.2	0.1	0	...	0		
B. leprae—G	0.001	0.006	0.008	0.01	.....	0.05	0.1	....	....	0	0.1	0	...	...	0	...	...	0.2		
B. leprae—H	0.001	0.006	0.03	.....	.....	0.05	0.1	....	....	....	0.1									
B. leprae — Hansen (from skin)	0.001	0.006	0.006	0.05	0.1	0.05	0.03	0.2	0.2	0.1	0.2	0	0.2	0	0	0.2	0	0.2	0	
B. leprae—Kedrowsky	0.002	0.004	0.008	0.006	0.01	0.03	0.05	0.05	0.03	0.03	0.1	0.2	0.1	0.1	0.1	...	0.2	0.2	0.2	
B. leprae — Duval (nonchrome)	0.002	0.004	0.006	0.006	.....	0.03	0.05	....	....	....	0.1	...	0.1	...	0.1	0.1				
B. tuberculosis — human	0.0005	0.002	0.004	0.006	0.008	0.03	0.03	0.03	0.03	0.03	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2
B. tuberculosis — bovine	0.001	0.004	0.004	0.008	.....	0.05	0.03	....	....	0	0.1	0.2	0.1	...	0.1	0.2	0.2	0.2		
B. tuberculosis — avian	0.001	0.002	0.006	0.006	.....	0.01	0.05	....	....	....	...	0	...	...	...	...	...	0.2		
B. tuberculosis — turtle	0.001	0.004	0.05	0.01	.....	0.03	0.05	....	....	....	...	0.2	...	...	...	...	0	0.2		
B. smegmatis	0.0005	0.004	0.004	0.006	.....	0.03	0.03	0.03	....	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.2			
B. of butter	0.001	0.006	0.008	0.01	.....	0.03	0.1	....	....	0	...	0	0.2	...	0	0.2				
B. of timothy	0.001	0.006	0.006	.....	.....	0	0.05	....	....	....	...	0	...	...	0.2					
B. typhosus	......	.....	0	.....	.....	0	0	....	....	....	0	...	0	...	...	0	0			
B. diphtheroid	......	0.05	0	0.1	.....	0	0	....	....	0	0	...	...	...	...	0	0			
Lipoid (Wassermann)	0.03	0.05	0	0	0	0	0	0	0	0	0	0	0	.03	0	0	0	0	0	0

0 means no fixation with 0.2 c c.

An examination of the results of the tests with the leper serums and the various antigens reveals several notable features that may be briefly summarized as follows: All serums give positive fixation with one or more of the acid-fast antigens, although there is a marked difference in the concentration of the fixation bodies in the different cases. In about half the serums, for example, fixation is obtained with less than 0.1 c c — one having a titer of 0.0005 c c — while the remainder required 0.1 or 0.2 c c to give a positive result. The serums of higher titer give the acid-fast group reaction with all the acid-fast antigens; but those of low titer fail to give fixation with certain of the organisms. Of the antigens, certain ones fix with every serum tested, and have a tendency to give fixation with somewhat smaller amounts of serum than the others. Those organisms that possess a higher antigenic value with leper serums are the same strains that show a similar superiority with rabbit serums immunized against acid-fast bacteria. About half the serums were tested with antigens from two nonacid-fast organisms, in order to determine the possible presence of nonspecific fixation. Two of the serums tested, both of which belong to the group having a higher fixing titer with the acid-fast antigens, gave fixation also with one of the nonacid-fast organisms. All other serums tested were negative. With the simple alcoholic Wassermann antigen, three serums were positive; two of these have the highest titer with acid-fast antigens of any of the group, while the third is of relatively low titer.

In general, the leper serums closely resemble the serums of rabbits immunized with acid-fast organisms in their behavior with bacterial antigens in the fixation test, and this is more especially true of those leper serums that have a high titer. Although there is some difference in the titer of each serum with the different antigens, leper serums tend to fix more uniformly with all acid-fast antigens than do the rabbit serums. In other words, the group reaction or acid-fast fixation is even more evident with serum from lepers than in immune rabbit serums. This illustrates the futility of attributing any etiologic significance to an acid-fast organism grown from a leper merely because that particular organism gives a positive fixation with leper serum. Such inferences made by certain workers have obviously been due to a lack of proper control experiments with other acid-fast antigens. Additional evidence of this is given by the fact that an antigen of acid-fast bacilli obtained from the skin lesions of a case of leprosy reacts less well with leper serums than some of the saprophytic acid-fast organisms (e. g., B. smegmatis).

The nonspecific fixation that occurred in two of the serums with a nonacid-fast antigen resembles the similar nonspecific reactions found with immune rabbit serums of high titer and is further evidence that such a phenomenon may be common to many serums having a high antibody content. In leper serums such fixation is given only with many times the minimal fixing dose of serum for acid-fast antigens and only with serums of high titer. By the titration of a serum, therefore, nonspecific reactions are readily recognized. The chief feature of interest in such reaction is that they may occur with certain naturally immunized human serums, as well as with artificially immunized rabbit serums.

TABLE 8

COMPLEMENT FIXATION WITH LEPER SERUMS AND HUMAN TUBERCLE BACILLI ANTIGEN

Cases	Minimal Fixing Dose of Serum (c c)	Type	Remarks
1	0.0005	Skin	Terminal stage of extensive general involvement; died a few months later
2	0.002	Skin	Actively progressing lesions
3	0.004	Mixed	Long duration; arrested
4	0.006	Skin	Active extending lesions
5	0.008	Skin	Long duration; stationary
6	0.03	Skin	Well advanced; progressing
7	0.03	Nerve	Active lesions; deformities; trophic ulcers
8	0.03	Skin	Several years duration; inactive
9	0.03	Skin	Short duration; improving
10	0.03	Skin	Long duration; improving
11	0.1	Skin	Advanced stage; improving
12	0.1	Nerve	Advanced stage; arrested
13	0.1	Nerve	Long duration; arrested
14	0.1	Mixed	Acute exacerbation; lepra fever
15	0.1	Nerve	Boy in incipient stage
16	0.2	Mixed	Improving; clinically well few months later; discharged
17	0.2	Nerve	Long duration; arrested
18	0.2	Nerve	Long duration; arrested
19	0.2	Nerve	Active anesthetic type
20	0.2	Nerve	Early anesthetic type

The relationship of the type of disease to the concentration of fixation substances in leper serum is graphically shown in table 8. In this table the minimal fixing dose of serum with human tubercle bacilli antigen only is given, since this antigen is one of those which reacted best with all serums. It is evident that the serums with the highest titer are from lepers with the nodular type of the disease, and that the cases in which the nerves alone are affected tend to have less antibody in the serum. The skin lesions of the nodular variety contain enormous numbers of acid-fast bacilli, while the purely anesthetic cases show as a rule relatively few organisms. It is of interest that the amount of fixation substances in the serum corresponds in general with the number of acid-fast organisms in the lesions.

In discussing the reaction of leper serums with lipoidal antigen, I realize that the series studied is small and that the percentage of positive reactions obtained is less than the percentage of Wassermann reactions reported by most other observers in lepers. One significant feature, however, deserves mention. The two serums of the highest fixation titer with acid-fast reactions, gave positive reactions with the lipoidal antigen. The resemblance of this lipoidal reaction in serums of high titer and the nonspecific fixation in such serums with other antigens seems more than a coincidence. A certain number of positive Wassermann reactions found in lepers are presumably due to a coincident syphilitic infection. By control studies on nonlepers two authors (Fletcher, Sordelli and Fischer) find about 10 per cent., while the only other recorded observation (Baermann and Netter) shows 25 per cent. in individuals (Chinese) showing no signs of leprosy. It is likely that a titration of serum with both lipoidal and acid-fast antigens would show a higher titer with the former in syphilitic lepers. Such a difference in titer was noted in the third positive case (case 10) observed in this series and suggests that in this instance a syphilitic infection may be the cause of the positive reaction rather than leprosy. As mentioned earlier, however, the proportion of positive Wassermann reactions reported in lepers is higher than can be explained by coincident syphilis, and must depend on some additional factor. The present experiments indicate that a positive fixation with leper serums and lipoidal antigen is obtained with those cases having a high concentration of fixation antibodies with acid-fast organisms, and that this is related to the nonspecific fixation found in serums of high titer with a number of antigens. It seems probable, therefore, that, in addition to the positive Wassermann reaction occurring in syphilitic lepers, an additional number of lepers whose serum contains a high concentration of leprous antibody give a nonspecific reaction with lipoidal antigen. It is significant in this connection that in the reports from the literature (table 1) a very much higher percentage of positive Wassermann reactions has been noted in cases of nodular leprosy, and it is in this type that the concentration of fixing bodies is greatest.

That certain serums have a rather high concentration of fixation antibodies, and that these serums react best with a number of antigens, may have some relation to the reports of fixation with leprosy serum and various antigens that have been reviewed in the early part of this paper. Since practically all of the observers used 0.1 c c of serum in the tests, it is quite probable that the leper serums that reacted positively were those with higher concentrations of complement-fixing antibodies. It will be remembered that as the observations were grouped on the basis of the type of antigen used, an increasing percentage of positive tests accompanied the use of antigens approaching acid-fast bacillary emulsions, the highest percentage being given with the latter type of antigen. This may be explained by assuming that a gradually increasing number of the serums having a lower concentration of antibodies react with such acid-fast antigens or, in other words, give a specific acid-fast fixation. The fact that every serum of the group studied in these experiments gave a positive acid-fast reaction was unexpected, and probably due in part to the somewhat more delicate methods of fixation used. The additional advantage of having several antigens of the group tested that have uniformly been capable of giving fixation with small amounts of serum, may also have been a factor. Since the concentration of fixing bodies is so low in some of the serums studied as to require 0.2 c c for a positive test, it is quite probable that if the series had been amplified certain serums would have been found containing still fewer of these antibodies, so that even 0.2 c c of serum would contain an insufficient amount to give a positive reaction. There is considerable evidence to indicate that this variation in antibody content of different leper serums is one of the important factors on which depends the difference in the number of positive results reported by various observers and is probably quite independent of the type of antigen used.

SUMMARY

Leper serums contain complement-binding substances that react with antigens of acid-fast bacilli and give an acid-fast fixation similar to that obtained with serums of rabbits immunized with acid-fast organisms. Some serums contain these antibodies in rather high concentration, notably those from cases with the nodular type of the disease; other serums show a relatively low antibody content. The serums of high titer may give a nonspecific fixation also with non-acid-fast antigens and with lipoidal (Wassermann) antigen, but only in comparatively low dilutions. It is suggested that this attribute of such high titer leper serums may explain a certain percentage of positive Wassermann reactions described in leprosy. Among the acid-fast antigens studied, certain organisms have superior antigenic properties in complement-fixation tests with leper serums. The acid-fast reaction given by leper serums with acid-fast bacterial antigens prevents the use of the complement-fixation reaction in obtaining evidence of the etiologic importance of any acid-fast organism isolated from leprosy.

BRIEF DESCRIPTION OF LEPROSY CASES

1.—Nodular type; Louisiana Leper Home. Old colored man with general infection of skin most marked on face and hands, little nerve involvement. Disease was in terminal stage and progressed rapidly to fatal outcome a few months later.

2.—Nodular type; University of California Dispensary. An Armenian, 40 years old, with actively progressing tubercular skin lesions of unknown duration.

3.—Mixed type; Louisiana Leper Home. Middle-aged white man with leprosy of long duration but not virulent in type. Evidences of the disease in both nerves and skin, but little progression in either.

4.—Nodular type; University of California Dispensary. White boy, 19 years; with actively extending erythematous lesions generally distributed. Duration, 8 months.

5.—Nodular type; Louisiana Leper Home. An old man with skin lesions of long duration. Disease apparently stationary.

6.—Nodular type; University of California Dispensary. A young Chinese with extensive skin lesions of several months' duration.

7.—Nerve type; Louisiana Leper Home. A young white man with active nerve lesions; trophic ulcers, deformity and mutilation.

8.—Macular type; Louisiana Leper Home. A young white man with macular lesions of several years' duration. The disease has not advanced beyond the incipient stage and is improving.

9.—Macular type; Louisiana Leper Home. An old white man with macular skin lesions of short duration; disease is in incipient stage and improving.

10.—Nodular type; Louisiana Leper Home. Young white man with advanced skin lesions. Condition is improving.

11.—Macular type; Louisiana Leper Home. Incipient case with macular lesions of skin. Disease is improving.

12.—Nerve type; Louisiana Leper Home. A middle-aged white man with nerve lesions of more than 15 years' duration. Disease apparently arrested.

13.—Nerve type; Louisiana Leper Home. A middle aged white man with trophic changes of long duration. The disease is still somewhat active.

14.—Mixed type; Louisiana Leper Home. A middle-aged white woman with both skin and nerve involvement of one year's duration. When the blood was taken the disease was virulently progressive and in a stage of acute exacerbation (lepra fever) that lasted 5 months. On subsidence of fever and inflammatory reaction of lesions, there was considerable improvement.

15.—Nerve type; University of California Dispensary. A boy, 15 years old, with lesion involving right ulnar nerve and its distribution in arm and hand.

16.—Mixed type; Louisiana Leper Home. Middle-aged white man in incipient stage with anesthesia of ulnar nerve distribution and infiltrated patches on skin of face, neck and arms. Patient was improving when specimen was taken and in few months all clinical and microscopic evidence of the disease had disappeared and patient was discharged from the Home.

17.—Nerve type; Louisiana Leper Home. A middle-aged white man with trophic lesions of more than 30 years' duration. The disease is apparently arrested after considerable mutilation and deformity of the extremities.

18.—Nerve type; Louisiana Leper Home. Same class as case 17.

19.—Nerve type; Louisiana Leper Home. Middle-aged colored man. Active anesthetic type with evidences of active degeneration of nerves.

20.—Nerve type; University of California Dispensary. A Japanese man, 32 years old, with diffuse infiltrating placque of 8 months' duration covering left cheek and temple with anesthesia of lesion and surrounding area.

BIBLIOGRAPHY

Akerberg, H.; Almkvist, J., und Jundell, I.: Weitere Beobachtungen über Wassermann's Serumreaktion bei Lepra, Lepra, 1910, *9*, p. 79.

Babes, V.: Ueber spezifische Reaktionen bei Lepra, Ztschr. f. Immunitätsforsch. u. exper. Therap., 1910, *7*, p. 578.

Babes, V., et Busila, V.: L'extrait éthéré de lépromes gardés depuis des années dans l'alcool comme antigène lépreux, Compt. rend. Soc. de biol., 1909, *61*, p. 817.

Babes, V., et Busila, V.: Etude sur les rapports qui existent entre les antigènes et les anticorps syphilitiques, tuberculeux et lépreux, Compt. rend. Soc. de biol., 1910, *62*, p. 181.

Babes, V., et Busila, V.: L'extrait éthéré des bacilles acidorésistants comme antigène, Compt. rend. Soc. de biol., 1910, *62*, p. 91.

Baermann, G., und Netter, N.: Die Wassermann-Neisser-Bruckche Reaktion in den Tropen, München. med. Wchnschr., 1910, *57*, p. 2131.

Bates, L. B.: Wassermann Test in the Tropics, Arch. Int. Med., 1912, *10*, p. 470.

Biehler, R., und Eliasberg, J.: Komplementbindung bei Lepra mit Leprösem Antigen, Lepra, 1910, *9*, p. 207; Deutsch. med. Wchnschr., 1911, *37*, p. 304; abst., Centralbl. f. Bakteriol. (Ref.), 1911, *50*, p. 154.

Bayon, H.: The Culture and Identification of the Germ of Leprosy, Tr. Soc. Trop. M. and Hyg., 1912, *5*, p. 158.

Bloomberg, H. D.: The Wassermann Reaction in Leprosy, Syphilis and Yaws, Philippine J. Sc., 1911, *6*, p. 335.

Bruck, C., und Gessner, E.: Ueber Serumuntersuchungen bei Lepra, Berl. klin. Wchnschr., 1909, *46*, p. 589.

Castelli, E.: The Wassermann Reaction, Jour. Am. Med. Assn., 1909, *53*, p. 936.

Clegg, M. T.: The Absence of the Luetin Reaction on Lepers Showing a Positive Wassermann Reaction, U. S. Pub. Health Bull., 1913, No. 61, p. 11; abst., Trop. Dis. Bull., 1914, *3*, p. 191.

Clegg, M. T.; McCoy, G. W., and Hollmann, H. T.: Immunity Studies on Leprosy, U. S. Pub. Health Bull., 1914, No. 66, p. 3.

Chujo, L., and Asakura, T.: Upon the Serological Diagnosis of Leprosy, Urol. and Cutan. Rev., Tech. Suppl., 1916, *4*, p. 12.

Currie, D. H., and Clegg, M. T.: Studies on Leprosy, XVI, Immunity, U. S. Pub. Health Bull., 1911, No. 50; Lepra, 1912, *13*, p. 10.

Duval, C. W., and Gurd, F. B.: Studies on the Biology of and Immunity Against the Bacillus of Leprosy, Arch. Int. Med., 1911, *7*, p. 230.

Ehlers, E. J., et Bourret, G.: La réaction de Wassermann dans la lèpre, Lepra, 1909, *11*, p. 368; Bull. Soc. path. exot., 1909, *2*, p. 520; abst., Bull. de l'Inst. Pasteur, 1910, *8*, p. 64.

Eitner, E.: Ueber den Nachweis von Antikörpern im Serum eines Leprakranken mittels Komplementablenkung, Wien. klin. Wchnschr., 1906, *19*, p. 1555.

Eitner, E.: Zur Frage der Anwendung der Komplementbindungsreaktion auf Lepra, Wien. klin. Wchnschr., 1908, *21*, p. 729.

Eliasberg, J.: Komplementablenkung bei Lepra mit syphilitischem Antigen, Deutsch. med. Wchnschr., 1909, *35*, p. 1922; abst., Centralbl. f. Bakteriol. (Ref.), 1910, *45*, p. 533.

Fleming, A.: A Simple Method of Serum Diagnosis of Syphilis, Lancet, 1909, *2*, p. 1512.

Fletcher, W.: The Wassermann and Luetin Reactions in Leprosy, J. Hyg., 1915, *15*, p. 102.

Fox, H.: The Wassermann and Noguchi Complement Fixation Test in Leprosy, Am. Jour. Med Sc., 1910, *139*, p. 725; Lepra, 1912-1913, *13*, p. 107.

Frugoni, C.: Studien uber das Blutserum der Tuberkulösen und died Exsudate der serösen Höhlen mittelst Komplementbindung, Berl. klin. Wchnschr., 1909, *46*, p. 1724.

Frugoni, C.: Syphilis und Lepra, Arch. f. Dermat. u. Syph., 1909, *95*, p. 223; abst., Centralbl. f. Bakteriol. (Ref.), 1910, *45*, p. 553.

Frugoni, C., und Pisani, S.: Vielfache Bindungseigenshaften des Komplementes einiger Sera (Leprakranker) und ihre Bedeutung, Berl. klin. Wchnschr., 1909, *46*, p. 1530.

Gaucher, E., et Abrami, P.: Le sérodiagnostic des formes atipiques de la lèpre, Bull. et mém. Soc. méd. d. hôp. de Par., 1908, 3. s., *25*, p. 497; Lepra, 1909, *8*, p. 152.

De Haan, J.: Over het voorkomen van antistoffen in het bloedserum van lijders aan lepra, Geneesk. Tijdschr. v. Nederl. Indie, 1909, *49*, p. 151; abst. Bull. de l'Inst. Pasteur, 1910, *8*, p. 64.

De Haan, J., en Grijns, G.: Over het voorkomen van de Wassermann die reactie bij lijders aan lepra, Geneesk. Tijdschr. v. Nederl. Indie, 1910, I, f. 4; Abst. Bull. de l'Inst. Pasteur, 1911, *9*, p. 75.

Inouye, S.: Ito, T., und Nakano, H.: Ueber die Serodiagnostik bei Lepra, Jap. Ztschr. f. Dermat. u. Urol., 1909, *9*, p. 544.

Jeanselme, E.: Cytologie et Sérologie de la Lèpre, Presse méd., 1912, *20*, p. 629; Lepra, 1912-1913, *13*, p. 112.

Jeanselme, E., et Vernes, A.: Réaction de Wassermann et réaction d'Eitner chez les syphilitiques et les lépreux, Bull. Soc. path. exot., 1915, *8*, p. 259.

Jundell, I.; Almkvist, J., und Sandman, F.: Wassermann's Syphilisreaktion bei Lepra, Centralbl. f. innere Med., 1908, *29*, p. 1181; abst., Lepra, 1909, *8*, p. 236.

Krefting, R.: Leichensera und die Wassermannche Syphilisreaktion, Deutsch. med. Wchnschr., 1910, *36*, p. 366.

Kritchewsky, J., und Bierger, O.; Zur Frage über Verhältnis des Bacillus leprae Hansen zu einigen bei Lepra gezüchteten Mikro-organismen, Ztschr. f. Hyg. u. Infectionskrankh., 1913, *73*, p. 509.

Lagane, L., et Columbier, P.: Formule sanguine de lépreux séjournant en France, Bull. Soc. path. exot., 1913, 6, p. 418.

Masslakowetz, P. P., und Liebermann, J. J.: Zur Frage über die Identität der Antigene, Arch. biol. nauk., 1908, 14, p. 2; abst., Centralbl. f. Bakteriol. (Ref.), 1909, 44, p. 490.

Mathis, C., et Baujean, R.: Le réaction de Wassermann dans la lèpre, Bull. Soc. path. exot., 1915, 8, p. 252.

Meier, G.: Serologische Untersuchungen bei Lepra, Lepra, 1910, 11, p. 334.

Mezinescu, D.: Maladie lépreuse des rats et lèpre humaine, Compt. rend. soc. de biol., 1909, 66, p. 56.

Möllers, B.: Serologische Untersuchen bei Leprösen, Deutsch. med. Wchnschr., 1913, 39, p. 595; Lepra, 1913-1914, 14, p. 161.

Montesano, D., et Sotiriadès, D.: La séro-réaction de Wassermann dans 48 cas de lèpre, Presse méd., 1910, 18, p. 659; abst., Lepra, 1910, 12, p. 182.

Müller, R., und Suess, E.: Vergleichende serologische Untersuchungen bei Tuberkulöse und Syphilis, Wien. klin. Wchnschr., 1911, 24, p. 559.

Nakajo, S., and Asakura, T.: The Serologic Diagnosis of Leprosy, J. Infect. Dis., 1915, 17, p. 388; identical with Chujo, S., and Asakura, T.: Urol. and Cutan, Rev., Tech. Suppl., 1915, 3, p. 296.

Nishiura, K.: Ueber die Komplementbindungsreaktion bei Lepra, Ztschr. f. Immunitatsforsch. u. exper. Therap., 1910, 7, p. 721.

Noguchi, H.: The Serodiagnosis of Syphilis, Jour. Am. Med. Assn., 1909, 53, p. 934.

Photinos, G., et Michiélidès, N.: La séro-réaction de Wassermann et la cuti-réaction de Pirquet dans la lèpre, 1912, 12, p. 207.

Pasini, A.: Sulla reazione della deviazione dèl complimento nella lepra, Osp. magg. Riv. scient. prat. d....di Milano, 1909, 4, p. 113.

Racamora, J. P.: Le salvarsan dans la lèpre: son influence sur le Wassermann dans cette maladie, Lepra, 1912-1913, 13, p. 4.

Récio, A.: La réaction de Wassermann dans la lèpre, San. y benefic. Bol. ofic. Habana, 1909, 2, p. 292; Lepra, 1909, 9, p. 178; abst., Bull. de l'Inst. Pasteur, 1910, 8, p. 63.

Schmitt, L. S.: On the Relation Between Rat and Human Leprosy, Univ. of Cal. Pub. Path., 1911, 2, p. 29.

Schmitter, F.: Leprosy in Its Relation to Treponematous Disease, Mil. Surgeon, 1914, 34, p. 311; abst., Trop. Dis. Bull., 1914, 3, p. 493.

Schüffner, W.: Die Wassermann'sche Reaktion bei Ulcus tropicum und der Wert der verschiedenen Antigene in den Tropen, Ztschr. f. Hyg. u. Infectionskrankh., 1912, 72, p. 362.

Serra, A.: La siero-diagnosi di Wassermann nella lepra, Policlin., Sez. med., 1909, 16, p. 545.

Slatinéanu, A., et Damélopolu, D.: Réaction de fixation dans la lèpre en employant la tuberculine comme antigène, Compt. rend. Soc. de biol., 1908, 60, u. 530.

Slatinéanu, A., et Daniélopolu, D.: Sur la présence d'anticorps spécifiques dans le sérum des malades atteints de lèpre, Compt. rend. Soc. de biol., 1908, 60, p. 309; Centralbl. f. Bakteriol., I, O., 1909, 48, p. 480.

Slatinéanu, A., et Daniélopolu, D.: Réaction de fixation avec le sérum et le liquide céphalo-rachidien des malades atteints de lèpre en présence de l'antigène syphilitique, Compt. rend. Soc. de biol., 1908, 60, p. 347; Centralbl. f. Bakteriol., I, O., 1909, 49, p. 289.

Slatinéanu, A., et Daniélopolu, D.: Prèsence de fixateur dans le liquide céphalo-rachidien des malades atteints de lèpre, Compt. rend. Soc. de biol., 1908, 60, p. 702; Centralbl. f. Bakteriol., I, O., 1909, 49, p. 288.

Sordelli, A., y Fischer, G.: Investigaciones serológicas en la lepra, Rev. Asoc. med. argent., 1916, 25, p. 558; Conf. Soc. sud. am. de hig., 1916-1917, 1, p. 117.

Steffenhagen, K.: Ueber Komplementbindungsreaktion bei Lepra, Berl. klin. Wchnschr., 1910, 47, p. 1362.

Sugai, T.: Zur klinisch-diagnostischen Verwertung der Komplementbindungsmethode bei Lepra, Arch. f. Dermat. u. Syph., 1909, 95, p 313; abst., Centralbl. f. Bakteriol. (Ref.), 1909, 44, p. 360.

Thomsen, O., und Bjarnhjedinson, S.: Untersuchungen über Komplementbindung mit dem Serum Aussätziger, Ztschr. f. Immunitätsforsch. u. exper. Therap., 1910, 7, p. 414; Lepra, 1910, 9, p. 191.

Wechselmann, W. und Meier, G.: Wassermannche Reaktion in einem Falle von Lepra, Deutsch. med. Wchnschr., 1908, 34, p. 1340; Lepra, 1909, 8, p. 226.

Wills, F. F.: The Relationship of Acid-Fast Bacilli, Centralbl. f. Bakteriol., I, O., 1912, 61, p. 37.